Advanced Polymerase Chain Reaction Module

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Polymerase chain reaction (PCR) is a widely used technique for amplifying DNA signatures. It is used in many biodetection systems such as LLNL's Autonomous Pathogen Detection System (APDS). To create a field deployable system, the current PCR reactors have to be miniaturized in terms of size, weight, reagents, and detection times.

This project describes the implementation of an advanced PCR module with an integrated polyimide heater and an integrated temperature sensor. An older version of this PCR module is currently an integral component of the APDS and ASTEP programs. The current PCR module uses a series of discrete resistors to heat a thermal mass during the PCR cycle. However, these discrete resistors and the temperature sensor are mounted on the outside of the thermal mass. The heater and the temperature sensor can be integrated into a polyimide flex cable and mounted inside the thermal mass near the reagent tubing. This implementation will improve the thermal efficiency and temperature sensor reading. This new PCR module will improve the performance of the APDS and ASTEP programs by improving the reliability and minimizing the power requirements of the module. These programs are vital to the Laboratory's mission in homeland security.

Project Goals

This project implemented a fully characterized PCR module with integrated polyimide heaters and temperature sensors. The characterization will focus on measuring the thermal performance of each module and the thermal stability as a function of time.

Relevance to LLNL Mission

The need for advanced PCR modules that allow for the detection of biological and chemical agents is directly aligned with LLNL focus areas that outline key technology needed for biological security/defense of the nation. This project will enable a robust, reliable PCR module that is field deployable in systems such as the APDS. There is also a significant opportunity to produce intellectual property in novel integration and assembly techniques.

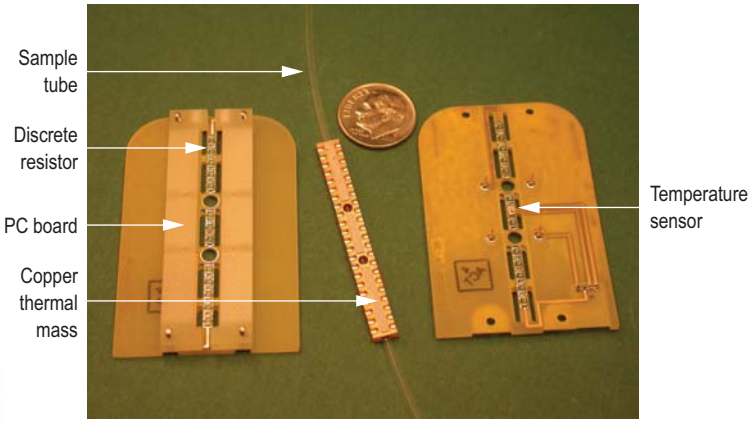

Figure 1. Previous version of the PCR module with discrete resistive heaters and temperature sensor.

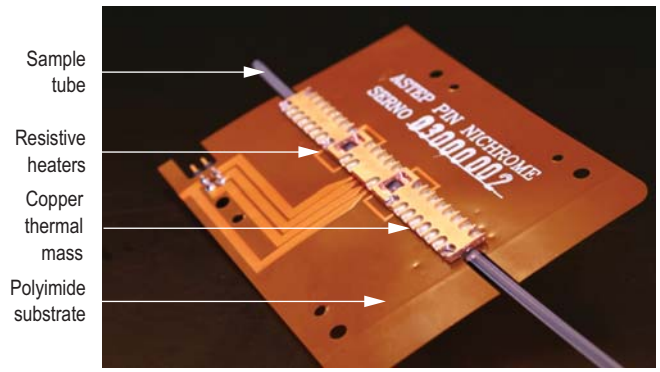

Figure 2. New version of the polyimide substrate for the PCR module with integrated resistive heaters and temperature sensor.

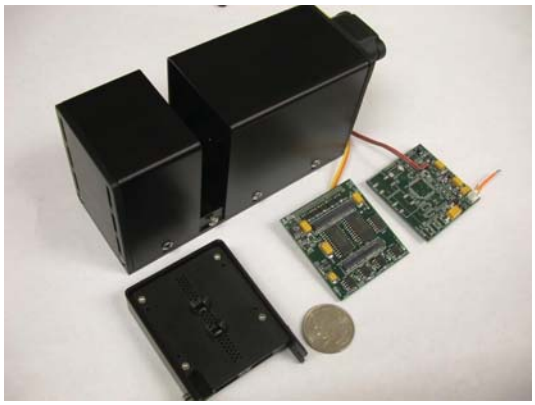

Figure 3. Complete PCR system with control electronics used to test the PCR module.

FY2007 Accomplishments and Results

The previous version of the PCR module is shown in Fig. 1. It consists of discrete resistors mounted to a printed circuit board. The temperature sensor is also mounted to the printed circuit board. The discrete resistors and the temperature sensor are mounted to the copper thermal mass that has a through hole for the reagent tube. The reactants for PCR are contained in this tubing and reactants are kept in the middle of the thermal mass to provide a uniform temperature to the reactants.

The previous version of the PCR module has several failure modes including delamination of the discrete resistors from the thermal mass and a varying temperature offset from the temperature sensor since it is mounted away from the reagent tubing. In order to correct these issues, the discrete resistors were replaced with a high-resistance, thin-film nickel alloy laminated on polyimide. Further, the temperature sensor was mounted onto the polyimide substrate as well to place it in contact with the reagent tubing. The polyimide substrate with integrated resistive heating elements and temperature sensor was fabricated (Fig. 2).

The integrated heaters on the polyimide substrate were tested for long-term reliability, and passed successfully. The temperature sensor was also integrated onto the polyimide substrate and tested successfully. This polyimide substrate was then integrated into the PCR system for testing and evaluation. The complete PCR system is shown in Fig. 3.

For the first test, two positive controls and one negative control were pumped into the reagent tubes. Forty-five PCR cycles were run to verify that the PCR was successful. As expected, signals from the positive controls increased with each PCR cycle and the negative control signal remained flat. Next, a target DNA molecule was inserted into the sample and a new test was performed. Again, the test was successful. The signal from the positive control increased with each cycle as well as the target signal. The negative control performed as expected.

Upon the successful completion of these tests, this technology was transferred to the APDS and ASTEP programs, and these programs are currently integrating this technology into their systems.

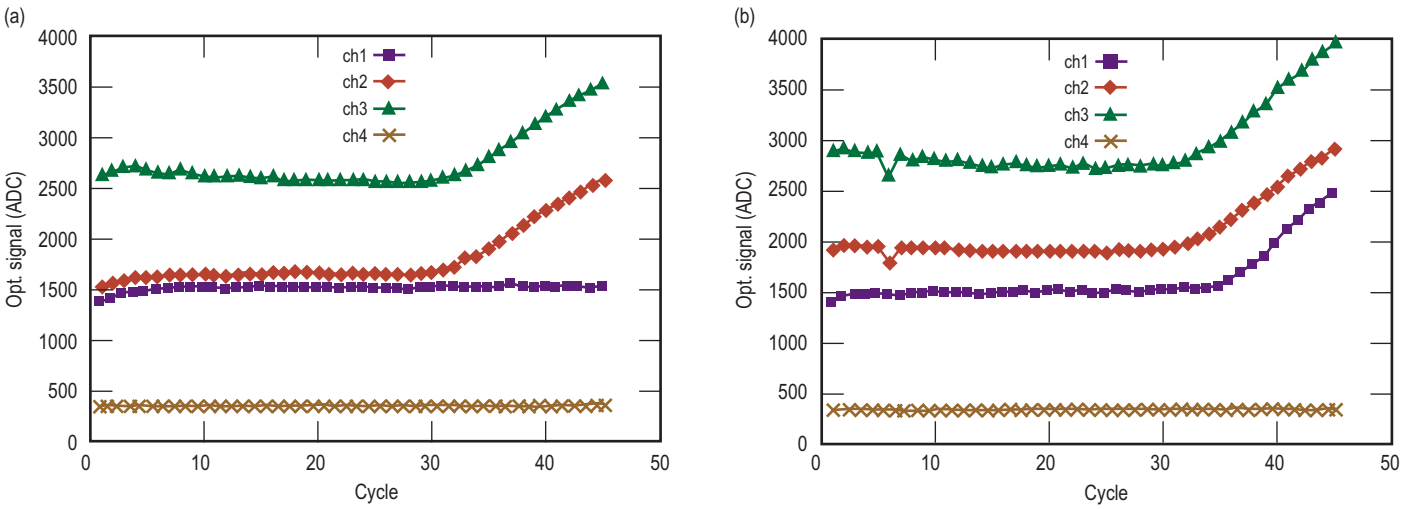

Figure 4. (a) Performance of the PCR module with only control samples and no target; (b) performance of the PCR module with the controls and a target.